degradation peak may appear as a single peak or be partially resolved showing a shoulder or two overlapping peaks.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

If the system suitability parameters have been met, then proceed as described in §436.216(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of mupirocin per milligram of sample as follows:

$$\text{Micrograms of mupirocin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the mupirocin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the mupirocin peak in the chromatogram of the mupirocin working standard;

P_s=Mupirocin activity in the mupirocin working standard solution in micrograms per milliliter;

C_u=Milligrams of mupirocin sample per milliliter of sample solution;

m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *pH.* Proceed as directed in §436.202 of this chapter using a saturated aqueous solution.

(4) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(5) *Identity.* Proceed as directed in §436.211 of this chapter, using the sample preparation method described in §436.211(b)(2).

[55 FR 2641, Jan. 26, 1990; 55 FR 11110, Mar. 26, 1990; 55 FR 14378, Apr. 17, 1990]

§455.50 Calcium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin is the calcium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 840 micrograms per milligram, expressed in terms of novobiocin on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 10 percent.

(iv) Its pH in a saturated aqueous suspension containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(vi) It demonstrates a positive color identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, specific rotation, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 5 milliliters of absolute ethyl alcohol and then dilute with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of 1,000 micrograms (estimated) per milliliter. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using a saturated aqueous suspension prepared by suspending 25 milligrams of calcium novobiocin per milliliter.

(5) *Specific rotation.* Proceed as directed in §455.51a(b)(8).

(6) *Identity.* Proceed as directed in §455.51(b)(7).

(7) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 43 FR 9801, Mar. 10, 1978; 50 FR 19921, May 13, 1985]

§ 455.51 Sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin is the monosodium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 6.0 percent.

(iv) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its residue on ignition is not less than 10.5 percent and not more than 12.0 percent, calculated on an anhydrous basis.

(vi) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(vii) It demonstrates a positive color identity test.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification: samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, specific rotation, identity and crystallinity.

(ii) Samples required on the batch; 10 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter, calculating on the basis of an anhydrous sample weight.

(6) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(7) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should be developed in the benzene layer.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.51a Sterile sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*